A Comparison of LC/MS Mass Analyzers for Screening, Confirmation and Quantification of Drugs in Blood

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John M. Hughes[1], Greg Kilby[2], Michael C. Zumwalt[3], Jeri D. Ropero-Miller[4], Peter R. Stout[4], and H. Chip Walls[5]
Agilent Technologies ([1]Pleasanton, CA; [2]Wilmington, DE; [3]Englewood, CO); [4]RTI International, Research Triangle Park, NC; [5]U. of Miami School of Medicine, Miami, FL

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Introduction

The information required for forensic and clinical toxicology ranges from initial screening through high-confidence confirmation and quantification, and all of these can be obtained using API LC/MS. Whole blood often is the most difficult fluid matrix for the analysis of drugs. This study analyzed the same set of blood extracts from actual medical examiner and DUID cases over a period of three days on five different LC/MS systems: single quadrupole, triple quadrupole, 3-D ion trap, Accurate-Mass TOF and Accurate-mass Q-TOF, using identical LC conditions on all the systems. In addition, the postmortem samples were analyzed on a TOF-MS equipped with a DART™ (Direct Analysis in Real Time) interface, for comparison to the LC/MS systems.

Examination of the resulting data shows the advantages and disadvantages of each type of mass analyzer for the analysis of low levels of drugs and metabolites in a complex biological matrix, and compares their abilities for both qualitative and quantitative analysis.

Experimental

Samples and Study Design

- Deuterated internal standards for all target analytes, added before sample preparation
- Calibrators and controls prepared in blank blood for target list of analytes
- Sample preparation one week before analysis using positive-pressure SPE (SPEware); final eluates evaporated and shipped frozen to LC/MS laboratory; reconstituted just before analysis.
- Post-mortem samples (RTI): one cocaine-positive, one methadone-positive.
- DUID case samples: benzodiazepine-positive
- Identical LCs, columns and conditions on all LC/MS systems
- Data acquisition modes

  Single quad: SIM

  QQQ: MRM, two transitions per analyte, qualifier ion ratios

  Ion Trap: AutoMS/MS to MS^3

  TOF: scan

  QTOF: scan and targeted MS/MS
- Data analysis

  quantification on SQ, QQQ, TOF, QTOF.

  identification/confirmation:

  ion trap (library search)

  TOF (accurate-mass database search with ret. Time)

Experimental

LC Parameters

Agilent 1200 Series RRLC system with binary pump SL, high-performance wellplate sampler, and thermostatted column compartment

Column: Zorbax Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm (Agilent Part#: 959764 -902)

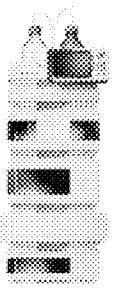
Col. temp: 50 C

Mobile phase:
A: Water + 5mM AmFormate + 0.05 % Formic Acid
B: Acetonitrile + 0.05 % Formic Acid

Flow rate: 250 µL/min

Gradient:

Time (min):	%B
1.0	5
6.0	40
8.0	95



Stop time: 10 min; Post time: 2 min.

Inj. vol.: 5 µL (SQ, QQQ & Trap)

2 µL (TOF)

0.1 µL (QTOF; adjusted based on prior results)

MS Parameters (Agilent systems)

Source: electrospray; ionization mode: positive

Capillary: 3000 V
Dry gas: 10 L/min, 350 °C
Neb. pres.: 30 psi

MS Parameters (JEOL AccuTOF-DART™)

(Postmortem samples only)
DART™ ion source, positive mode
Ring lens voltage: 5 V
Orifice 1 voltage: 20 V
Orifice 2 voltage: 5 V
Electrode 1: 150 V
Electrode 2: 350 V
Temperature: 300°C
Detector optimized at 2,200 V
Calibration w/ PEG prior to each sample run

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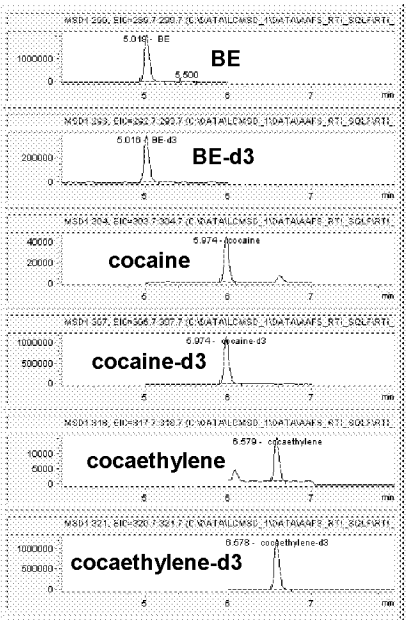
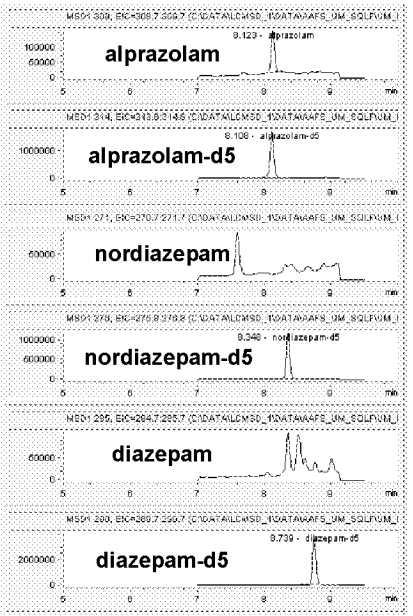
Results and Discussion

6140 Quadrupole LC/MS

- SIM for sensitivity and selectivity down to low ng/mL

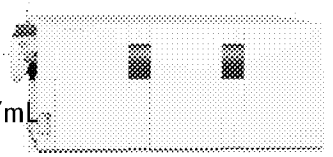


RTI postmortem cocaine sample:
Benzoylecgonine 1253 ng/mL
Cocaine 8.8 ng/mL
Cocaethylene 2.7 ng/mL

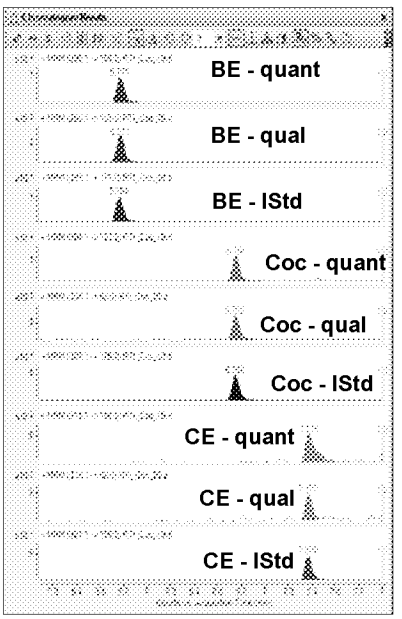


U Miami DUID case sample:
Alprazolam 5.6 ng/mL
Diazepam, nordiazepam < LOD

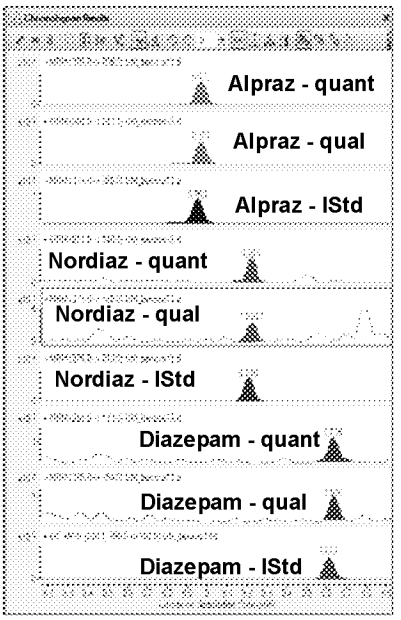
6410 Triple Quadrupole LC/MS/MS



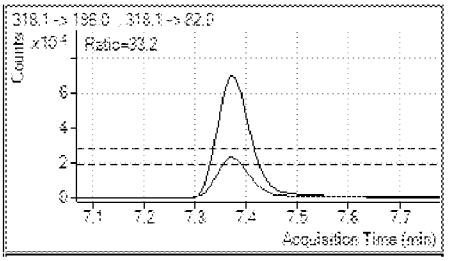
- MRM with two transitions for high confidence confirmation along with quantification
- Accurate and precise quantification below 2 ng/mL



RTI postmortem cocaine sample:
Benzoylecgonine 1448 ng/mL
Cocaine 1.1 ng/mL
Cocaethylene 0.1 ng/mL



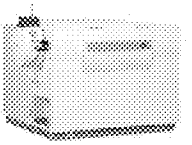
U Miami DUID case sample:
Alprazolam 0.8 ng/mL
Diazepam, nordiazepam present, < LOQ

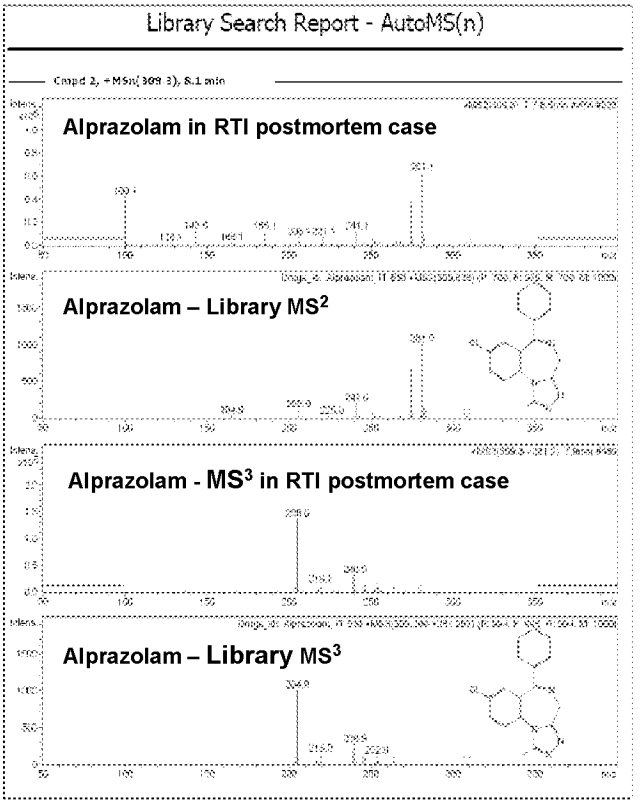


Overlaid quant and qualifier EICs

6330 Ion Trap LC/MS^n,

- Best scan sensitivity
- AutoMS^n for screening and confirmation
- MS^2 and MS^3 spectra for library search w/ drug library





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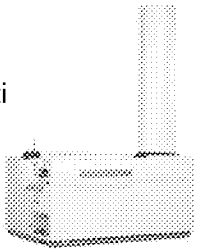
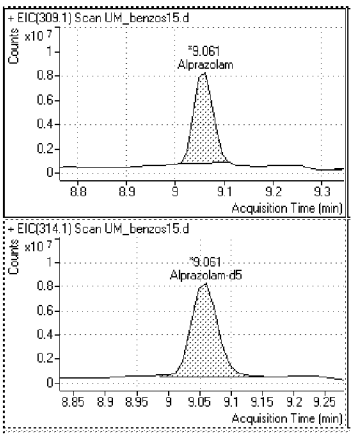
Results and Discussion

6210 Accurate-Mass TOF LC/MS

- Mass accuracy < 2 ppm for screening/confirmation/identification
- Narrow-mass EIC's for sensitive and selective quantification



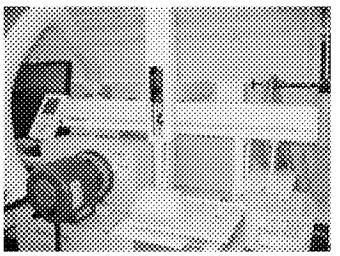
U Miami DUID case sample:
Alprazolam 2.7 ng/mL
with excellent S/N
(EIC mass width ± 10 ppm)

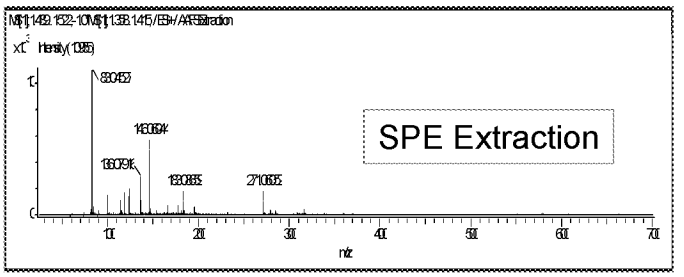


TOF-DART™

With CTC-PAL autosampler

- Not all drugs found in postmortem samples
- Cleanup and concentration required to detect ng/mL

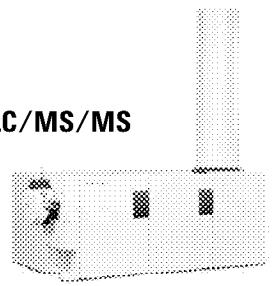


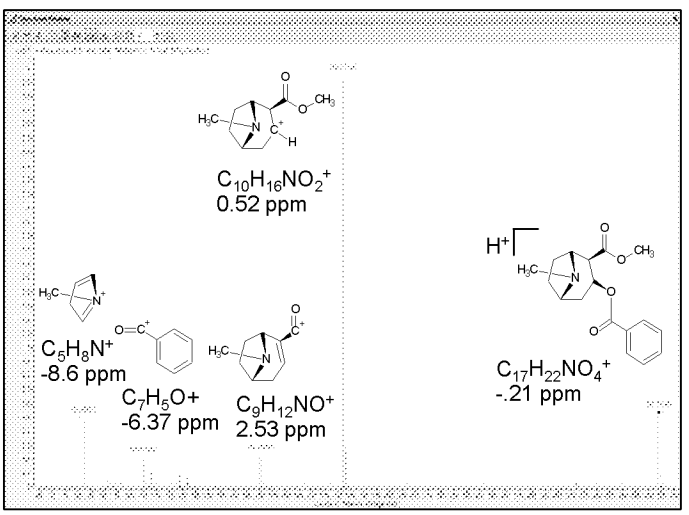


Found: Cocaine, EME, BE, Oxycodone, Propoxyphene, Norpropoxyphene and Alprazolam

6520 Accurate-Mass Q-TOF LC/MS/MS

- MS/MS with accurate mass
- MS ≤ 2 ppm
- MS/MS ≤ 5 ppm





Accurate-mass MS/MS spectrum of cocaine
Structures proposed from literature: P-P Wang and M G Bartlett, J. Mass Spectrom. **33**, 961-967, (1998)

Conclusions

The original question which led to this study was: "What type of LC/MS is the "best" instrument for forensic toxicology?"

• Sensitivity
- All 5 LC/MS instruments in this study were able to detect and quantitate at the lowest calibrator levels (5 ng/mL)
- DART-TOF has limited sensitivity for biological samples, but great for drug chemistries

•Quantification
- QQQ has best overall sensitivity and specificity, uses ion ratios for high confidence confirmation as well
- SQ is next best at quant for sensitivity and linearity
- TOF has at least equivalent sensitivity to SQ, better in specificity with routine accurate mass
- Ion trap is most sensitive to co-eluting interferences and least easy for quantification.
- QTOF used in this study can do good quant in MS or MS/MS, but is the most expensive of instruments in the study

•Identification and Confirmation
- Ion trap has the best full-scan sensitivity, can use both MS/MS and MS^3 spectra with library search.
- TOF has fast-scan accurate mass to identify compounds from formula databases (plus retention time), rather than spectral libraries
- QTOF can also confirm structure with accurate-mass MS/MS
- Both TOF and QTOF can look for untargeted compounds not originally sought, from accurate-mass scan data without re-running the sample.